Table 2. Competition of deoxyribonucleoside triphosphates for the enzyme-DNA complex binding site. The experimental system is as given for Table 1 with nonlabeled triphosphates added as indicated. Sephadex G-200 fractions I and II provide the source of enzyme. Fraction I (0.1 mg) is assayed with denatured primer and fraction II (0.05 mg) is assayed with native primer.

Nonlabeled triphosphate added (6)	Amount (μmole)	Binding (nmole ^{3}H-nucleotide mg^{-1} protein fraction)	
		Fraction I	Fraction II
	^{3}H-TTP (0.02 μmole)		
		17.31	14.73
TTP	0.04	9.64	8.07
dATP	0.04	10.32	9.12
dCTP	0.04	11.70	8.17
dGTP	0.04	9.89	7.64
dATP, dCTP, dGTP each	0.02	8.52	6.94
	^{3}H-dCTP (0.02 μmole)		
		16.43	13.91
TTP	0.04	9.39	8.47
dATP	0.04	10.16	8.18
dCTP	0.04	8.68	6.84
dGTP	0.04	10.71	7.29
dTTP, dATP, dGTP each	0.02	7.94	5.82
	^{3}H-dATP (0.02 μmole)		
		18.45	15.75
TTP	0.04	11.78	8.48
dATP	0.04	10.12	7.27
dCTP	0.04	12.13	9.39
dGTP	0.04	10.56	7.89
TTP, dCTP, dGTP each	0.02	8.42	6.74

larly decreased by the addition of any of the four unlabeled triphosphates (Table 2). It appears from the competition experiments that binding requires between 0.03 and 0.04 μmole of triphosphate to saturate the amount of enzyme protein used in the assay. With 0.02 μmole of ^{3}H-TTP (6) and 0.01 μmole of nonlabeled TTP, the same number of counts is bound as with 0.02 μmole of ^{3}H-TTP alone. If 0.02 μmole of nonlabeled TTP is added, only slight inhibition of binding was found; it required 0.04 μmole of nonlabeled TTP to reduce binding by approximately one half. Similar amounts are required for competition with the other deoxyribonucleoside triphosphates.

Differences exist in the time course of the incorporation and binding reactions and in the relation to the amount of DNA primer. The incorporation reaction remains linear for at least 60 minutes; maximum binding is reached after 15 minutes of incubation and remains constant for up to 60 minutes. Binding increases linearly as the DNA in the system is increased to 150 μg with enzyme remaining constant at 0.2 mg of *p*H 5.0 protein fraction per 0.5 ml of reaction mixture. Incorporation, however, is linear only up to 50 μg of DNA and does not increase with increases in DNA. It is not possible at present to estimate the number of binding sites on an enzyme molecule since the enzyme preparations are not sufficiently pure.

The process of binding of deoxyribonucleoside triphosphates to a DNA–DNA polymerase complex can be dissociated from polymerization of nucleotides into DNA. The two reactions differ with respect to primer DNA preference and to time required for the completion of the reaction. Kornberg (7) postulated that *Escherichia coli* DNA polymerase molecules have five active sites, one of which binds triphosphates. When a correct base pair is within the active site, the enzyme can respond, presumably by a conformational change, so that the catalytic step may proceed. If an incorrect triphosphate were found, it would be rejected and no conformational change would occur. It is premature to suggest a similar postulate for mammalian DNA polymerase. It is still possible that two different protein molecules are involved in these two reactions, one acting as a nucleoside triphosphate acceptor and the other as a polymerase enzyme. In studying the triphosphate binding to DNA polymerase alone by equilibrium dialysis, Kornberg concluded that the four common nucleoside triphosphates compete for a single site (7). Our data with mammalian enzyme and DNA are in agreement with this finding.

PETER OVE*
JOHN LASZLO
Department of Clinical Pharmacology and *Medicine, Duke University Medical Center, Durham, North Carolina 27706*

References and Notes

1. R. Mantsavinos, *J. Biol. Chem.* **239**, 3431 (1964).
2. G. A. Bray, *Anal. Biochem.* **1**, 279 (1960).
3. Hokin's solution (935 ml of ethanol, 60 ml of glacial acetic acid, and 4 ml of 2*M* NaOH).
4. P. Ove, J. Laszlo, M. D. Jenkins, H. P. Morris, *Cancer Res.*, in press.
5. P. Ove, O. E. Brown, J. Laszlo, *ibid.*, in press.
6. Abbreviations are: dATP, deoxyadenosine triphosphate; dCTP, deoxycytidine triphosphate; dGTP, deoxyguanosine triphosphate; TdR, thymidine; TMP, thymidine monophosphate; TTP, deoxythymidine triphosphate; and UTP, uridine triphosphate.
7. A. Kornberg, *Science* **163**, 1410 (1969).
8. We thank M. D. Jenkins and O. E. Brown for technical assistance. Supported in part by PHS grant (CA 08800-03) and American Cancer Society grant (P363D).
* Present address: Department of Anatomy and Cell Biology, University of Pittsburgh Medical School, Pittsburgh, Pennsylvania 15213.

1 May 1969; revised 14 July 1969

Sex Attractant of Female Dermestid Beetle Trogoderma inclusum Le Conte

Abstract. *Two components of the sex attractant of a female dermestid beetle* Trogoderma inclusum *Le Conte were identified. They are (−)-14-methyl-*cis*-8-hexadecen-1-ol and (−)-methyl-14-methyl-*cis*-8-hexadecenoate. Other* Trogoderma *species also respond to the compounds.*

We report here the isolation, identification, and synthesis of two components of the sex attractant of a female dermestid beetle, *Trogoderma inclusum* Le Conte (1). This beetle is a common pest in dried-milk factories, and it consumes a wide variety of products such as grain, cereals, dried milk, spices, nuts, and other stored, dried foods. A closely related beetle *T. granarium* Everts (the khapra beetle) is a very serious pest of stored cereal products in hot, dry parts of the world. The response of the male *T. inclusum* beetle to the unmated female has been described by Burkholder and Dick (2) whose bioassay was used to monitor the isolation steps.

Each of the two identified compounds is active by itself. Responses of the male beetle to the synthesized attractants and to the isolated compounds are identical; the males respond to the compounds in a manner similar to that of males exposed to paper disks that have been in contact with females (2). Females do not respond in any of these tests, nor do they respond to paper disks that have been in contact with males.

In the laboratory bioassay, a response from the male *T. inclusum* was elicited by as little as 0.001 μg of synthesized compound 1 (3 males responded out of a total number of 40 tested) or 0.01 μg of synthesized compound 2. The effect of a mixture of the two synthesized compounds was additive; no synergistic or blocking effects were detected, but cross-attractance was general in the genus. The responses of males of several other *Trogoderma* species to 1 μg of each of the synthesized compounds are shown in Table 1. Forty males were used in each test, each male being placed in a 3.7-ml shell vial. An assay disk containing 1 μg of the attractant was suspended 1 cm above each male for 1 minute. The response was considered positive when the behavior reported earlier (2) was observed.

The interspecific responses for five

species of male *Trogoderma* to the extracts of unmated females of seven species of *Trogoderma* have been reported (*3*). Samples of extract of unmated female *T. inclusum* were tested against the khapra beetle (*T. granarium*). The tests showed that extracts of female *T. inclusum* and *T. granarium* are equally attractive for *T. granarium* males. Two other attractant fractions were isolated from female *T. inclusum*, but they have not yet been identified. It seems likely from the cross-attractant study that some or all of the four compounds, or close analogs thereof, are involved in the sex attractants of other *Trogoderma* species.

To isolate the attractant components, 250,000 unmated female *T. inclusum* beetles, in batches of 40,000, were extracted with benzene (four 300-ml portions per batch) in a Waring Blendor. The solvent was separated by centrifugation and removed by distillation through a Vigreux column at 150 torr. The distillation residue (50 g) was distilled in 5-g batches in a short-path (2 cm) still onto a condenser (cooled with dry ice) at 65°C and 0.01 torr. Solutions of the distillate (15 g) in benzene were thoroughly extracted with ice-cold 0.1*N* sodium hydroxide. The solvent was removed at 150 torr, and the nonacidic material (9 g) was chromatographed in four portions on silica gel (Gallard-Schlesinger, 90 to 200 mesh, 100 g in a 3.5 by 20 cm water-cooled column); the solvents used successively were pentane (300 ml), diethyl ether (600 ml), and methanol (200 ml). The active fraction (5 g in the ether eluate) was fractionated by gas chromatography [5 percent SE-30 on Gas Chrom Q 60/80 mesh, 0.6 m by 7.5 mm (inside diameter) glass tubing; 160° to 180°C at 4°C per minute; 50 cm³ of He per minute]; the fraction eluting between 14 and 27 minutes was fractionated on a Carbowax 20 M column [5 percent on Gas Chrom Q 60/80 mesh, 1.7 m by 4 mm (inside diameter) glass tubing; 110° to 200°C at 2°C per minute; 32 cm³ of He per minute]. The fraction eluting between 30 and 32 minutes (about 1 mg from 100,000 beetles) was identified as compound 1 (see below). The fraction eluting between 21 and 27 minutes (about 3 mg from 100,000 beetles) was fractionated on a diethyleneglycolsuccinate column [5 percent HI-EFF-1BP (Applied Science) on Gas Chrom Q 60/80 mesh, 3 m by 4 mm (inside diameter) glass tubing; 151°C; 30 cm³ of He per minute]. The material eluting between 33 and 37 minutes (about 0.2 mg from 100,000 beetles) was identified as compound 2 (see below).

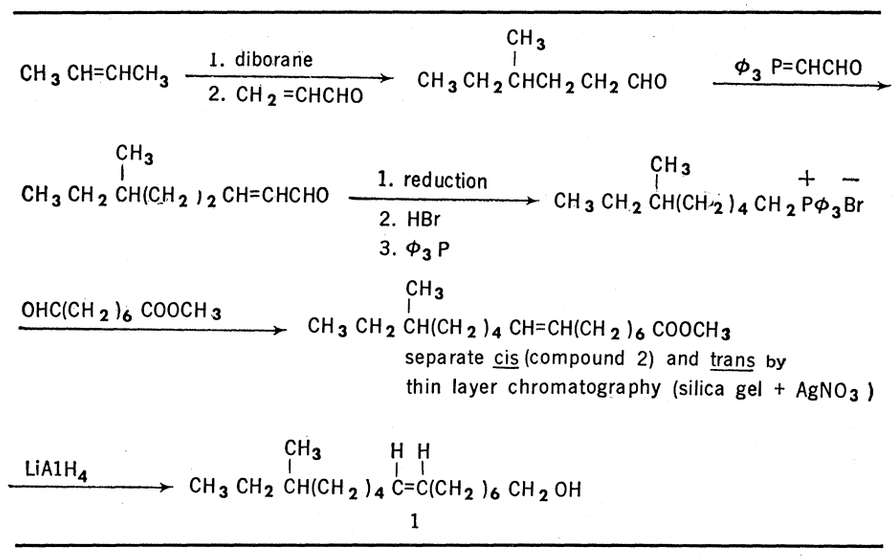
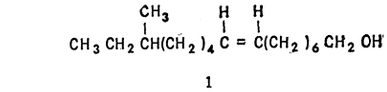


Fig. 1.

The infrared spectrum of compound 1 showed the following diagnostic peaks (CS_2, μ): 2.99 (OH), 9.45 (CH_2–OH), no peak near 10.4 (no *trans* double bond). The mass spectrum showed the following diagnostic peaks (mass to charge, *m/e*): 254 (molecular ion, M), 236 (M − H_2O). The molecular formula was assumed to be $C_{17}H_{34}O$, which accommodates one double bond or a ring. The nuclear magnetic resonance (NMR) spectrum (CCl_4, 100 Mhz τ) was: 4.80 (2, −HC=CH−, m), 6.49 (2, $-CH_2CH_2OH$, τ), 8.05 (4, $-CH_2CH=CHCH_2$, m), ~8.69 [20, $(CH_2)_9$, CH, OH], 9.14 (6, $2CH_3$, m). The optical rotation was minus (*4*). Therefore the partial structure is a C_{17}, branched-chain, unsaturated (*cis*), primary alcohol. Ozonolysis (*5*) showed that it was an aldehyde with a retention time between those of *n*-octanal and *n*-nonanal. Hydrogenolysis (*6*) revealed two major products that were identified by mass spectrometry as 3-methylpentadecane and 3-methylhexadecane. Compound 1, therefore, is (—)-14-methyl-*cis*-8-hexadecen-1-ol.

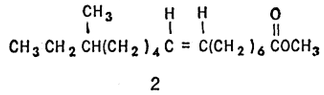


1

The infrared spectrum of compound 2 showed the following diagnostic peaks (CCl_4, μ): 5.74 (C=O), 8.00, 8.34, and 8.53 (CH_3OC=O), no peak near 10.4 (no *trans* double bond). The mass spectrum showed the following diagnostic peaks (*m/e*): 282 (M), 251 M − CH_3O), 250 (M − CH_3OH). The molecular formula was assumed to be $C_{18}H_{34}O_2$, which allows one double bond or ring besides the C=O group. Ozonolysis gave the same aldehyde that was obtained from compound 1. The optical rotation was minus (*4*). Therefore the structure of compound 2 is (—)-methyl-14-methyl-*cis*-8-hexadecenoate.

Table 1. Response of males of several *Trogoderma* species to the synthesized compounds (1 μg). Forty males were used in each test.

Males	Number responding	
	Compound 1	Compound 2
T. inclusum	31	12
T. simplex	23	5
T. glabrum	10	1
T. parabile	8	0
T. sternale	7	0
T. grassmani	30	0



2

Both compounds were synthesized by the sequence shown in Fig. 1.

Spectral data for the isolated and synthesized compounds were congruent, and the synthesized compounds were biologically active.

J. Otto Rodin
Robert M. Silverstein*
Life Sciences Division,
Stanford Research Institute,
Menlo Park, California 94025
Wendell E. Burkholder
John E. Gorman
Market Quality Research Division,
Agricultural Research Service,
U.S. Department of Agriculture, and
Department of Entomology,
University of Wisconsin,
Madison 53706

References and Notes

1. R. M. Silverstein, D. L. Wood, W. E. Burkholder, *Abstracts, 157th Meeting of the American Chemical Society*, Minneapolis, April 1969.
2. W. E. Burkholder and R. J. Dicke, *J. Econ. Entomol.* **59**, 540 (1966).
3. K. W. Vick, W. E. Burkholder, J. E. Gorman, *Ann. Entomol. Soc. Amer.*, in press.
4. Because of the small amount of material and the small rotation measured, no accurate optical rotation value is available.
5. M. Beroza and B. A. Bierl, *Anal. Chem.* **38**, 1976 (1966).
6. M. Beroza and F. Acree, Jr., *J. Ass. Offic. Agr. Chem.* **47**, 1 (1964).

7. Supported by the Agricultural Research Service, U.S. Department of Agriculture [contract 12-14-100-9500(51)]. We thank F. C. Church and L. D. Stietzel of Stanford Research Institute for the mass and NMR spectra, P. Lin and R. H. Iwamoto for the optical rotation, and Dr. H. Z. Levinson (Hebrew University, Israel) for testing the compounds against *T. granarium*. The beetles were reared by D. Clark, J. Coffelt, and R. Fries at the Stored Products Insect Laboratory, Fresno, California.

* Present address: Department of Chemistry, State University College of Forestry, Syracuse University, Syracuse, New York 13210.

12 May 1969

Paramagnetic Unit in Spinach Subchloroplast Particles: Estimation of Size

Abstract. *A pulsed ruby laser (wavelength, 694.3 nanometers) was used to measure the dependence on light intensity of light-induced electron paramagnetic resonance (EPR) signal I for short flashes of uniform duration (400 microseconds). Approximately 10^{18} photons per square centimeter per flash from the unattenuated beam were available to the sample of subchloroplast "system I" particles from spinach. The experimental dependence of the EPR signal height plotted as a function of the total number of incident photons per flash was exponential. From measurement of the slope at a very low relative photon flux and the saturated EPR signal amplitude, the value for the cross section or "effective size" of the light-induced paramagnetic unit, σ_{EPR}, was found to be 300×10^{-17} square centimeter. This result is compared with a measured optical absorption cross section, σ_{694nm}, of 2.5×10^{-17} square centimeter, for the identical sample at the laser wavelength. The hundredfold difference in size supports the thesis that the paramagnetic state is a property of an aggregate of chlorophyll molecules of the same general size as the photosynthetic unit.*

As early as 1932 Emerson and Arnold presented evidence that cooperative action of approximately 2400 chlorophyll molecules was required for the reduction of one molecule of CO_2 or the liberation of one molecule of O_2 (*1*). This idea of cooperative action has given rise to the concept of the "photosynthetic unit" whose effective area, or cross section, presented to incident light of a given wavelength, is consistently larger by at least two orders of magnitude than the effective area presented to light absorption by one chlorophyll molecule alone.

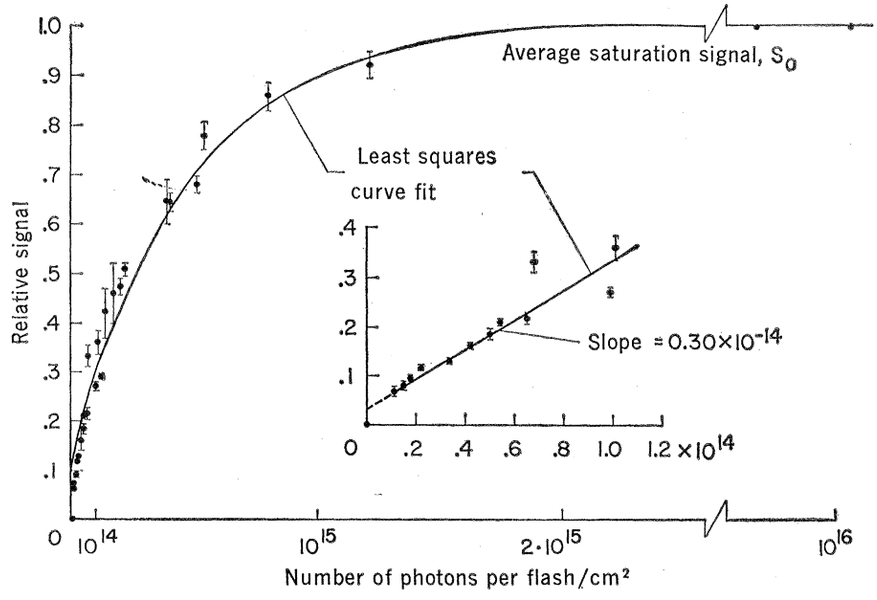


Fig. 1. Height of the induced EPR signal as a function of the number of photons per laser flash. The inset is an expanded portion of the lowest part of the curve. The solid curves are the result of a least-squares fit of all averaged data points including zero, treated with equal weight.

In a photosynthetic system, whether intact algae or chloroplast preparations, a light-induced paramagnetic state can be observed by the technique of electron paramagnetic resonance (EPR) spectroscopy. The precise identity and function of the photoprocess of the observed paramagnetic states have been under investigation (*2*). Evidence is accumulating for the assignment of the two distinctive EPR signals to photosystems I and II in the plant photosynthetic process. One piece of evidence for this assertion is the finding of Vernon *et al.* that particles could be isolated from spinach which represent an efficient photosystem I as it exists in the intact chloroplast with little contaminating inactive chlorophyll (*3*). They present a number of criteria for the activity of photosystem I, among them the facts that the spin intensity of signal I was considerably greater than that for intact chloroplasts, and that P_{700} and signal I exhibited similar behavior under a variety of conditions. These subchloroplast particles proved to be suitable material for a cross-section determination for the following reasons: an aqueous suspension of them in the EPR cuvette was optically thin and nonscattering; induction effects were absent; the rise time of the signal was less than a millisecond, while the paramagnetic intermediates were relatively stable, taking several seconds to decay in the dark. The suspension yielded reproducible results over several hours at room temperature.

A pulsed light experiment analogous to that of Emerson and Arnold can be performed on the paramagnetism in such particles, and from the resulting data a measure of the cross section for the process can be made.

The relation between the EPR signal and the incident number of photons is assumed to be

$$S(n) = S_0[1 - \exp(-\sigma \cdot n)]$$

with

$$\sigma = \frac{\left(\frac{\Delta S}{\Delta n}\right)_{n \to 0}}{S_0}$$

where

$$\left(\frac{\Delta S}{\Delta n}\right)_{n \to 0} = \text{initial slope}$$

n is the number of photons per square centimeter, σ is the cross section in square centimeters, $S(n)$ is the relative